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Fabrication of two flow phantoms for Doppler ultrasound imaging

Xiaowei Zhou, David A. Kenwright, Shiyang Wang, John A. Hossack, Jr., *Fellow, IEEE*, and Peter R. Hoskins

Abstract—Flow phantoms are widely used in studies associated with Doppler ultrasound measurements, acting as an effective experimental validation system in cardiovascular related research and in new algorithm / instrumentation development. The development of materials that match the acoustic and mechanical properties of vascular system are of great interest while designing flow phantoms. Although recipes that meet the flow phantom standard defined by International Electrotechnical Commission (IEC) 61685 are already available in the literature, standard procedure of material preparations and phantom fabrications have not been well established. In this study, two types of flow phantom, with and without blood vessel mimic, are described in details in terms of the material preparation and phantom fabrication. The phantom materials chosen for the two phantoms are from published phantom studies and their physical properties have already been investigated previously. Both flow phantoms have been scanned by ultrasound scanners and images from different modes are presented. These phantoms may be used in the validation and characterization of Doppler ultrasound measurements in blood vessels with diameter above 1 mm.

Index Terms—Ultrasound, vessel phantom, blood vessel mimic, wall-less phantom, tissue mimicking material, blood mimicking fluid.

I. INTRODUCTION

FLOW phantoms are used widely in the investigation of blood flow related measurements conducted by medical ultrasound. These phantoms are able to mimic the flow characteristics within blood vessels both for clinical ultrasound studies, where frequencies are in the range 2 – 15 MHz, and for the use in preclinical ultrasound where higher frequencies (15 – 60 MHz) are used [1, 2]. In order to ensure that the simulated ultrasound images have a high similarity to images from the human/animal body, it is important to make sure that the relevant physical properties of these flow phantoms match the properties of human/animal tissues. Although a large number of studies on arterial applications were implemented by Doppler ultrasound based on various flow phantoms [3-6], fabrication of

these phantoms and properties of the phantom materials have not been well documented.

It is possible to produce a real-vessel phantom based on the use of an excised artery [7] however such phantoms are of limited use as they require access to biological tissues and are not reproducible in terms of phantom geometry, composition and physical properties.

Flow phantoms for assessing Doppler ultrasound measurements include three materials: a Tissue Mimicking Material (TMM) which matches the acoustic properties of soft tissue, a Blood Mimicking Fluid (BMF) which matches the acoustic and viscous properties of blood, and a Vessel Mimicking Material (VMM) which matches the acoustic properties of the vessel wall. In addition, a pump is needed to controllably move the BMF in a flow loop. A wide range of mimicking materials have been investigated over several decades reviewed in Hoskins [8].

In 2001 the specifications of a standard flow phantom were defined in International Electrotechnical Commission (IEC) 61685 [10]. Recipes were available at that time for a suitable TMM [11] and BMF [12] whose properties match those required by IEC 61685. At that time there was no acoustically matching VMM. The best commercially available material was a rubber material (C-flex) which has a sound speed close to 1540 m.s^{-1} [8]; however, C-flex has an attenuation value that is some five to ten times higher than soft tissue. Thin-walled bifurcation flow phantoms have been constructed using silicone elastomer [9] however the resulting wall was not acoustically equivalent having an acoustic velocity of 1020 m.s^{-1} .

The lack of a suitable VMM led to the development of wall-less vessel phantoms in which the TMM is in direct contact with the BMF [13]. The advantage of a wall-less phantom is that it eliminates the impedance mismatch between the TMM and the wall, thereby removing artifacts in the Doppler measurements. While straight-tube wall-less phantoms could be constructed using this approach with the TMM, more complex three-dimensional (3D) geometries, such as a bifurcation, led to phantom failure with splitting of the TMM [14]. In addition splitting also occurred in straight-tube preclinical flow phantoms where the vessel was only a few mm below the surface [15]. To overcome this problem, a stronger TMM was formulated based on the use of two hydrogels, konjac and carrageenan [14]. Recently the konjac and carrageenan-based TMM (KC-based TMM) was acoustically characterized over the frequency range 5 – 60 MHz [1] and has been used in the construction of a preclinical flow phantom [15].

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Subsequently a recipe for a vessel mimic was formulated based on the use of PVA-c [16, 17]. The PVA-based vessel mimic has property values that almost match the IEC 61685 standards except for its low attenuation coefficient. PVA-based vessels can be embedded into gelatin- or agar-based tissue mimic to form a PVA-c vessel flow phantom [17 18], where all materials (TMM, VMM and BMF) have been formulated with property values close to IEC standard values.

As noted above IEC 61685 [10] provides specifications for the physical properties of the components of a standard flow phantom and candidate recipes. As described above a number of publications have been produced from different groups on candidate recipes and manufacturing procedures for the different phantom components. To date there is no agreed standard recipe and manufacturing protocol for the different flow phantom components. In this paper, we describe the fabrications of two different flow phantoms in detail, one without mimicking the blood vessel (wall-less flow phantom) and the other using PVA-c vessel mimic, based on the hands-on experience in our group for many years. These detailed procedures, together with previously proven recipes, aim to provide standard methods for making flow phantoms for Doppler ultrasound research.

IMPORTANT NOTICE: Readers are reminded that in the methods presented, toxic materials and small particles are sometimes employed. Ensure to wear suitable protective clothing and gloves and a functional fume hood must be used while weighing, decanting and mixing all these materials other than water.

It should be noted that some suppliers identified in this paper operate internationally and some do not. In many cases, local alternative suppliers can be found for those not operating internationally. Many of the larger international suppliers to the scientific community (e.g. Fisher Scientific, Sigma-Aldrich, etc.) sell the same products using identical, or similar, part numbers from nationally-based distribution systems. Also note that the acoustic and mechanical properties of materials used in paper were characterized at room temperature (20 – 22 °C) so that any work on flow phantoms should be performed in a lab with a similar temperature.

II. KC-BASED WALL-LESS FLOW PHANTOM

Konjac and carrageenan are two organic-based materials which when added to water in combination form a strong, flexible material. Scatterers in the form of metal powders of varying grades provide the appropriate attenuation and speckle pattern, and added glycerol ensures that the speed of sound matches that of tissue. It can be manufactured in a controlled manner with well-characterized, highly reproducible and uniform acoustic properties. The recipe of this KC-based TMM is given in Table I. This TMM's sound speed and attenuation factor were tested by Kenwright et al [1], which justified the application of this TMM both for clinical and pre-clinical studies. The KC-TMM had reported speed of sound of 1548 ± 3 m s⁻¹, and an attenuation (dB cm⁻¹) behaviour of $0.01024f^2 + 0.3639f$ where f is transmit frequency (MHz) [1].

A. Procedure for preparing KC-based TMM

Equipment required in preparing KC-based TMM is listed in Table II and detailed steps are described as follows.

- 1) Set water bath temperature to 90°C, and measure out the DI water in a container.
- 2) In the fume hood, measure out both types of aluminium oxide powders (0.3 µm and 3 µm) and the silicon carbide powder.
- 3) Use the domestic sieve, sift together the powders and mix well until evenly distributed. Then, sift the mixed powders into the DI water. Whisk for two minutes to remove any clumps.
- 4) Place the water-powder mixture in a vacuum pump for degassing. Release the vacuum if the air bubbles threaten to overflow the container, then re-apply the vacuum. Note that this procedure takes approximately 30 mins with a vacuum pressure of 4.8 MPa (700 psi). **REMARKS:** This procedure should be repeated until no further air bubbles form in the mixture.
- 5) Place the container in a water bath, insert overhead stirrer and place on the cover as shown in Fig. 1. Note that the temperature of the water bath at this point is not critical.
- 6) Switch on the stirrer to approximately two rotations per second. Note that the aim is to ensure that the particles mix without settling whilst preventing a large vortex, which

TABLE I
THE RECIPE FOR MAKING KC-BASED TMM

Component	Manufacturer	Weight (%)
DI water	---	84
Glycerol (99%)	G7757, Sigma-Aldrich Ltd, Dorset, UK	10
Silicon carbide (400 grain)	Logitech, Glasgow, UK	0.53
Aluminum oxide powder (Al ₂ O ₃) 3 µm, OCON-008	Logitech, Glasgow, UK	0.96
Aluminum oxide powder (Al ₂ O ₃) 0.3 µm, OCON-015	Logitech, Glasgow, UK	0.89
Konjac powder	FMC Biopolymer, Philadelphia,	1.5
Carrageenan powder commercial grade: Type 1	Sigma-Aldrich Ltd, Dorset, UK	1.5
Potassium chloride	Sigma-Aldrich Ltd., Dorset, UK	0.7

should be avoided to reduce the amount of air introduced to the mixture; if a vortex forms, reduce the rotation speed of the mixer. REMARKS: The container's internal diameter should closely match the width of the stirrer to prevent settling of the powders in the mixture.

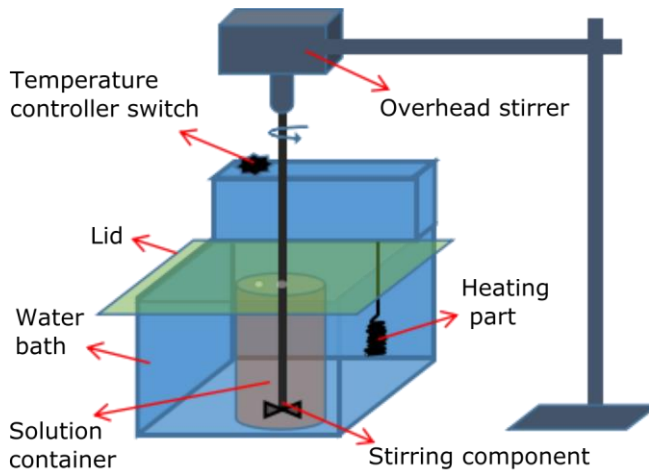


Fig. 1. Water bath and overhead stirrer for cooking and stirring mixture

- 7) Measure out the konjac and carrageenan powders in the fume hood. Then, using a sieve, sift together the konjac and carrageenan powders and mix well until evenly distributed.
- 8) When the temperature of water bath is greater than 60 °C, add the konjac and carrageenan powders to the water/powder solution through the opening hole (Fig. 1) in the water bath cover whilst the electronic stirrer continues mixing. These powders should be introduced slowly to prevent clumping. Add potassium chloride to the solution. REMARKS: These powders dissolve better at higher temperatures (approximately 60 °C).
- 9) Once the water bath reaches a temperature of 90 °C, the mixture should be heated at this temperature under continuous stirring for 1 h. By this time the konjac and carrageenan powders should have fully melted and be homogenous. REMARKS: a cover (better to use a thermally nonconductive cover) must be used to help achieve the high temperature in the water bath and to reduce the water evaporation in the TMM solution. The holes on the cover must be sealed as well.
- 10) Add the glycerol to the solution through the opening hole with the mixture continuing to be stirred for 10 more mins.
- 11) Switch off the heater in the water bath and allow the mixture to cool whilst continuing stirring. Once the temperature has reached approximately 80 °C, the container can be removed from the water bath and the TMM can be poured into the desired phantom mold. Note that the TMM mixture will set relatively quickly in room temperature conditions. REMARKS: Care should be taken to avoid pouring the TMM too slowly to prevent an uneven

distribution in the mold. It may be useful to warm the phantom mold prior to pouring the TMM. The TMM should also be poured in a single continuous motion to prevent the introduction of air bubbles into the phantom.

- 12) Once the TMM has cooled and set, a solution of 9% glycerol and 91% DI water should be poured over the phantom for acoustic coupling and to prevent the TMM from drying out.

TABLE II
EQUIPMENT FOR PREPARING KC-BASED TMM

Equipment	Details
Fume hood	--- (A). Water heater with thermometer. (TechneTE-10D thermoregulator; Techne Ltd. Duxford, Cambridge, UK) (B). Overhead electric controllable stirrer. (IKA EUROSTAR power control-visc overhead stirrer IKA Werke GmbH & Co. KG, Staufen, Germany) (C). Cover with hole for stirrer and a closable opening to allow introduction of ingredients
Water bath combining	
Scale	PCB 2500-2, Kern & Sohn GmbH, Balingen, Germany
Others	Domestic sieve, domestic whisk, container for mixing the TMM, disposable gloves, and the vacuum pump (the one shown in Table VIII)

B. Fabrication of KC-based wall-less phantom

Here we provide a detailed description for the fabrication of this wall-less flow phantom. The phantom consists of a plastic container with pipe connectors that can be connected via flexible tubing to a reservoir of BMF. A retractable metal rod provides a mold for the vessel, such that when the KC-TMM sets in the container and the metal is removed, a vessel cavity is left in the KC-TMM through which the BMF can be pumped. The sizes of the metal rod and the container determine the vessel cavity diameter and size of the phantom. Reticulated foam provides a matrix which anchors the KC-TMM to the pipe connectors, preventing leaking of the BMF.

The diagram to indicate the fabrication of this wall-less phantom is given in Fig. 2 and the detailed steps are given as follows.

- 1) The pipe connectors need to be attached to either end of the plastic container so that they are aligned horizontally. Drill holes in the plastic container at the required depth and glue the pipe connectors in place with the fast setting glue (e.g. epoxy from Araldite), such that the flexible tubing can be connected to the outside of the phantom and that the connector protrudes into the internal space of the phantom.
- 2) Glue a piece of the reticulated foam around the pipe connector. The depth of the reticulated foam should be similar to the protrusion of the pipe connector. REMARKS: The glue should not fill the depth of the foam, as the TMM will need to set in the foam cavities such that the foam acts as an anchor to hold the set TMM in place.
- 3) Insert the metal rod through both pipe connectors. Mark on the side of the container the desired fill height for the TMM above the metal rod. This will be dependent on the imaging depth of the ultrasound system being used. REMARKS: The greater the depth of TMM, the less prone to rupturing the phantom will be.
- 4) Prepare the KC-based TMM as described in Section A.
- 5) Pour the KC-based TMM into the phantom container to the required depth over the metal rod. REMARKS: It may be useful to warm the phantom container to prevent the TMM from setting too quickly.
- 6) Once thoroughly set and cooled, withdraw the metal rod slowly without twisting.
- 7) For applications where high frequency ultrasound is used, the BMF (described in following section) may need to first be circulated through a running gear pump (for example the GJ-N25 Micropump system given in Table VI) for about 10 mins. This is to break up any clumps of particles in the BMF before using it, for these clumps can introduce spikes in the Doppler signal due to reflections from trapped air.
- 8) Connect pieces of flexible pipe to the inlet and outlet of a pump system (such as those given in Table VI). REMARKS: The pump chosen in the vessel phantom must have an output range compatible with the corresponding application.
- 9) Note that it is necessary to ensure that all air is removed from the phantom. Before collecting data from this phantom, run the pump system for 10 minutes with a portion of BMF that is dedicated for removing bubbles in the system. REMARKS: It may be helpful to tap the pipes and rotate the pump system to assist in dislodging trapped air.
- 10) Once the air has been removed, the phantom is ready for use. REMARKS: A newly degassed BMF should be recirculated through the phantom for collecting data.

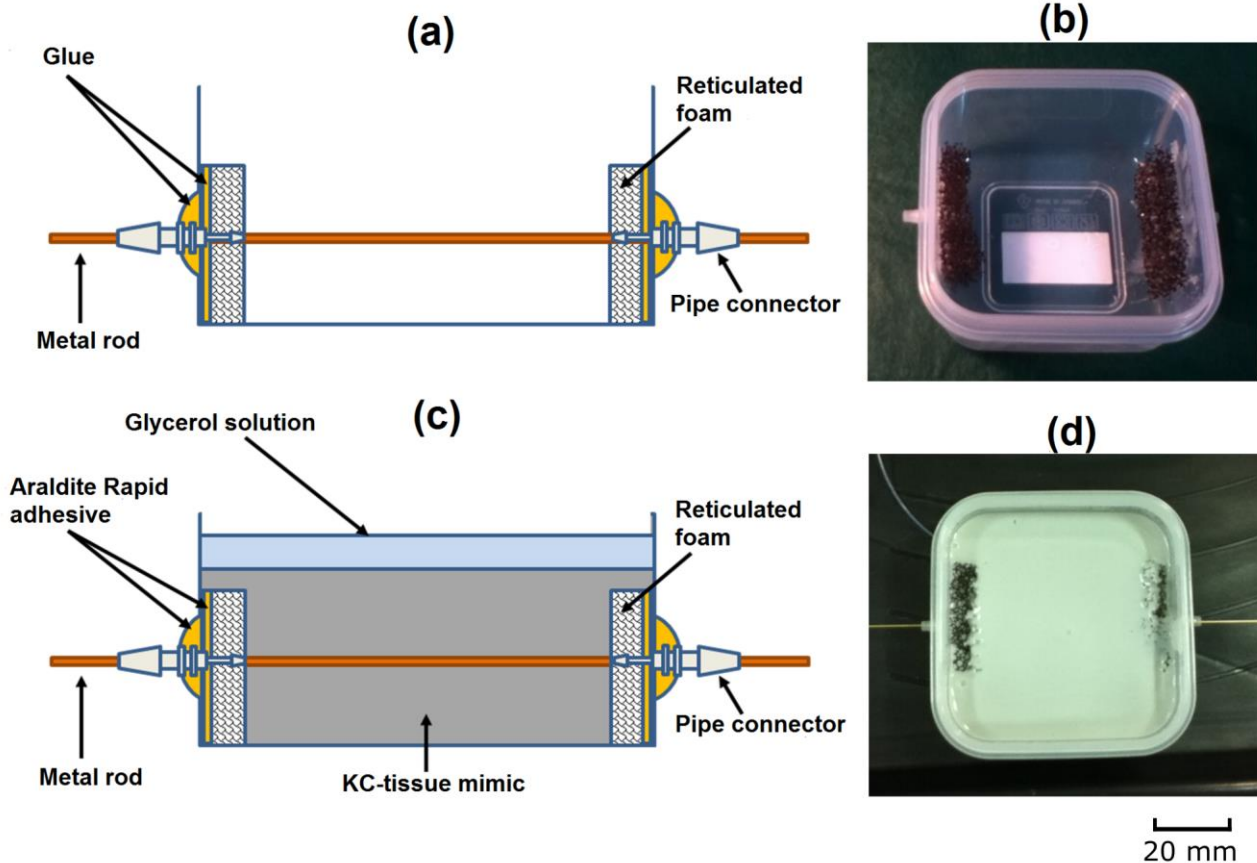


Fig. 2. Schematic illustrations and photographs of the KC-based wall-less phantom. (a) Schematic illustration and (b) photograph of empty vessel phantom mold ready for PVA solution pouring. (c) Schematic illustration and (d) photograph of vessel phantom (vessel diameter with 1 mm) with KC-TMM and glycerol solution

III. PVA-C VESSEL FLOW PHANTOM

Polyvinyl alcohol cryogel (PVA-c) is derived from a solution of polyvinyl alcohol (PVA) in water. After going through a few freeze-thaw cycles, a gel is formed, termed a cryogel due to the manufacturing process. PVA-c was initially used for mimicking blood vessels in MRI experiments and was later introduced to ultrasound phantoms. The acoustic and mechanical properties are controlled by the percentage of PVA used and the number of freeze-thaw cycles [16-19]. 10 wt% PVA with two freeze-thaw cycles was found to produce a vessel mimic having the sound speed of 1538 ± 5 m/s, attenuation of $0.07 \text{ dB cm}^{-1} \text{ MHz}^{-1}$ at central frequency 3.5 MHz and Young's elastic modulus of 79 ± 11 kPa [17]. Except for the low attenuation factor, this PVA-c vessel has a close match to the properties of healthy human arteries. In this procedure, the recipe recommended by King et al [20] was used, and is listed in Table III. Silicon carbide was added to the PVA to act as scatterers and to ensure that there was an acoustic signal on the B-mode image from within the vessel wall.

TABLE III.
THE RECIPE FOR MAKING PVA-C VMM

Component	Manufacturer	Weight (%)
DI water	---	88.79
Polyvinyl alcohol	Sigma-Aldrich Ltd, Dorset, UK	10
Benzalkonium chloride (BC)	Thermo Fisher Scientific, Geel, Belgium	0.46
Silicon carbide (400 grain)	Logitech, Glasgow, UK	0.75

The PVA-c vessel is embedded within TMM to form a vessel flow phantom. The agar-based TMM, which was firstly proposed by Teirlinck [11], was chosen in this procedure to mimic the soft tissue. The physical properties of this agar-based TMM were measured with sound speed of 1551 m.s^{-1} , attenuation coefficient of 0.53 at 4 MHz and density of 1058 kg.m^{-3} [11, 21]. The details of this recipe are given in Table IV.

A. Procedure for preparing PVA-c vessel mimic

The equipment used for making PVA-c vessel is listed in Table V and the procedure is described as follows.

- 1) Measure out the required amount of PVA powder, antibacterial agent Benzalkonium chloride (BC), Silicon carbide, and deionized water into a metal beaker. The BC is added to help the PVA-c vessel stay longer. Remember to perform the weighing in an operating fume hood.
- 2) Put the metal beaker in the water bath as shown in Fig. 1 and set the temperature at 100°C . REMARKS: Make sure the

- lid (including the opening hole) is well-sealed to prevent the water in the metal beaker from vaporization.
- 3) Keep the water bath heater on to heat the PVA mixture until the mixture in the bottle changes into a clear uniform purified condition. This procedure may take 2- 3 h.

TABLE IV.
THE RECIPE FOR MAKING AGAR-BASED TMM

Component	Manufacturer	Weight (%)
DI water	---	82.4
Glycerol (99%)	Sigma-Aldrich Ltd, Dorset, UK	11.32
Silicon carbide (400 grain)	Logitech, Glasgow, UK	0.53
Aluminum oxide powder (Al_2O_3) 3 μm : OCON-008	Logitech, Glasgow, UK	0.94
Aluminum oxide powder (Al_2O_3) 0.3 μm : OCON-015	Logitech, Glasgow, UK	0.88
Benzalkonium chloride (BC)	Thermo Fisher Scientific, Geel, Belgium	0.92
Agar	Thermo Fisher Scientific, UK	3

TABLE V.
EQUIPMENT FOR PREPARING PVA-C VESSEL MIMIC

Equipment	Details
Freezer	V145-40E, Peter Swan & Sons Ltd, Loanhead, Edinburgh, UK
Scale	PCB 2500-2, Kern & Sohn GmbH, Balingen, Germany.
Water bath combing	The same as the one in Table II but without overhead stirring
Others	Metal beaker, in-house designed vessel mold, beakers, and disposable gloves

- 4) After obtaining the clear PVA solution, take the metal beaker out from the water bath and wait until the hot PVA solution cools down to approximately 50 °C before injecting it to the vessel molds.
- 5) While making the PVA solution, an in-house designed vessel mold is prepared. The mold consists of three parts, a stainless-steel straight tube, a stainless-steel rod and a flange, as shown in Fig. 3. Different sizes of tube and rod can be obtained from any suppliers as long as they are stainless material. The flange was designed in Solidworks (Dassault Systemes, US) and manufactured in a mechanical workshop where Computerized Numerical Control Tools are available. The mold's size, indicated by the D, L, and Th in Fig. 3, can be chosen to make blood vessels with different inner diameter, length and wallthickness. The parts of the mold are washed clean and allowed to dry before use. There is a see-through hole near one end of the mold so that the PVA gel could be injected into the mold when all these three parts are fitted together. The two ends of the mold must be sealed with tack tape.

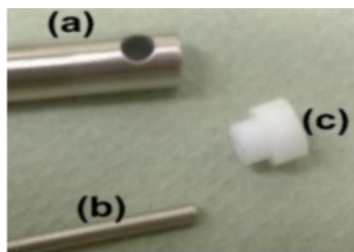


Fig. 3. Parts of the in-house designed vessel mold and its assembly diagram.

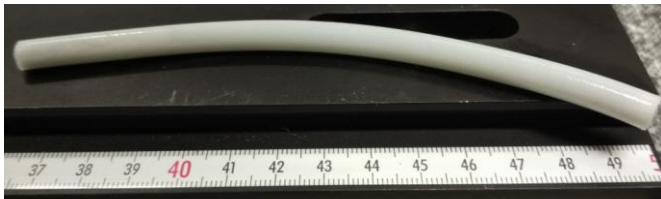


Fig. 4. The PVA-c vessel mimic

- 6) Once the mold and PVA solution are both ready, inject the PVA solution into the mold with a syringe carefully until the mold is thoroughly filled with PVA solution. Leave the mold at room temperature (22 ± 0.5 °C) for 10 h in an upright position (with the hole at the upper side) to let

bubbles come out. REMARKS: Inject the PVA solution into the mold with a proper speed before it cools down. A cooler (below 48 °C) PVA solution is too viscous to flow down to the mold.

- 7) Seal the see-through hole with tack tape and put the mold into a freezer. The mold is placed in the freezer at -20°C for 14 h, after which time the mold is taken out from the freezer and left for 10 h at room temperature (22 ± 0.5 °C). It would be preferable if a programmable freezing-oven could be used to control the freeze-thaw cycle. Two freeze-thaw cycles are suggested to obtain realistic properties as changing the number of freeze-thaw cycles will have an influence on the vessel properties [17].
- 8) After going through the freeze-thaw cycle, the PVA vessel mimic is carefully pulled out from the mold. This vessel is ready for use as shown in Fig. 4. This PVA vessel mimic must be store within water to prevent it from being dry when it is not in use.

B. Procedure for preparing agar-based TMM

The procedure and equipment used for making agar-based TMM are identical to that used for the KC-based TMM; there are some differences in chemicals as noted here. Replace the KC with agar powder; the potassium chloride is not needed for agar-based TMM. Note that the solution is needed to cook for about one hour at around 95 °C after adding all ingredients. During cooking, the overhead stirring system should be always on and the lid must be sealed to prevent vaporization. Once the agar-based solution cools down to 42 °C, it is ready to be poured into the desired phantom mold. As for KC-based TMM, care also should be taken when pouring this solution to the mold.

C. Fabrication of PVA-c vessel flow phantom

With the PVA-c vessel and the agar-based TMM ready, the detailed procedure for fabricating the PVA-c vessel flow phantom is given as follows.

- 1) Similar to the fabrication of the wall-less phantom, holes at either sides of the phantom container are drilled at the required depth and the pipe connectors are fixed in place with the fast setting glue (e.g. epoxy from Araldite), such that the flexible tubing can be connected to the outside of the phantom and that the connector protrudes into the internal space of the phantom.
- 2) Due to dehydration of PVA-c vessel, only the segment within the phantom container will be PVA-c vessel which is surrounded by agar-based TMM and injected with water inside while not in use. The rest of the loop will use other flexible tubes to form the complete circular loop. Connectors are required to connect the PVA-c vessel and the connecting tubes. The diagram to shown this phantom fabrication is given in Fig. 5.
- 3) Before pouring the agar-based TMM solution into the phantom container, the PVA-c vessel must be filled with water and seal both ends to have a constant water pressure inside to prevent the PVA-c vessel from being pressed into non-circular shape by the poured TMM.

- 4) Pour the previously prepared agar-based TMM into the container slowly to bury the PVA-c vessel at certain depths. REMARKS: To avoid the PVA-c vessel being melted by the hot TMM solution, wait until the TMM solution's temperature is around 42 °C.
- 5) Wait until the agar-based TMM sets at about 40 °C. REMARKS: Put 9% glycerol solution at the top of the TMM and inject this glycerol solution into inside the PVA-c vessel to store this fabricated phantom.

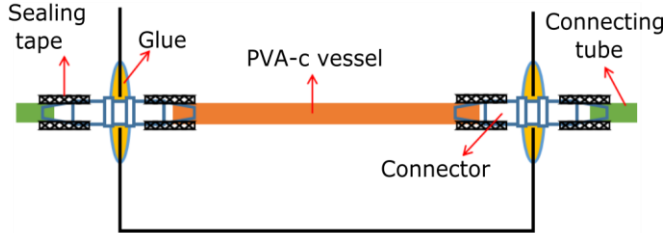


Fig. 5. Diagram to mount the PVA-c vessel onto the phantom container

IV. PUMP SYSTEM IN THE FLOW PHANTOM

A pump system is always required in the flow phantom to drive the fluid through the phantom loop, functioning similarly to the heart in the cardiovascular system. The specifications of a pump system, such as its output flow rate, precision and flexibility (controllable), should be carefully considered according to the specific applications.

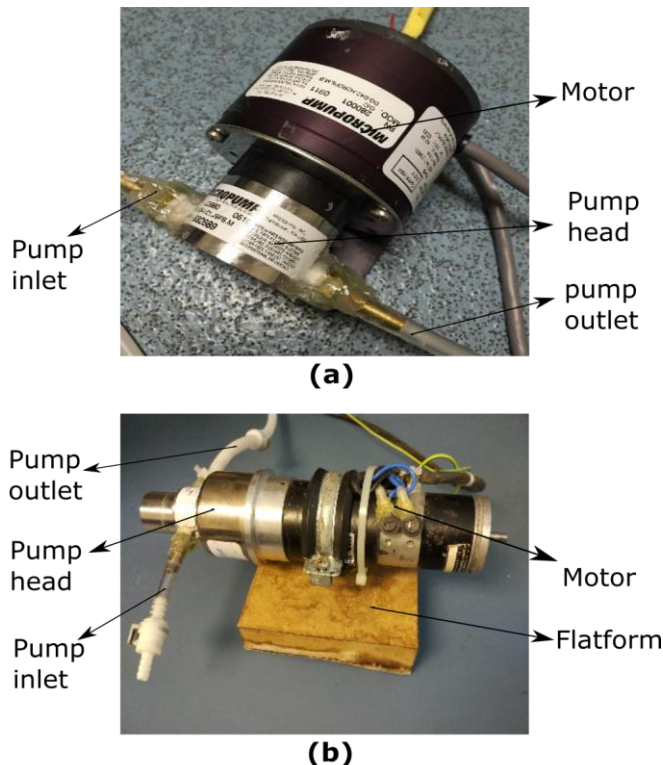


Fig. 6. Two types of gear pump. (a) The GA-V21 pump system; (b) the GJ-N25 pump system.

The pump system, consisting of a gear pump head fitted together with a motor, was used in the flow phantoms in this paper. The motor must be coupled with a magnetic cup mounted at the pump head. For some pump systems, the pump head and motor are already coupled together and ready to use after purchase, such as the GA-V21 pump as shown in Fig. 6a. It is also possible that the pump head and the motor are purchased separately. In this case, the magnetic cup must be fitted to the motor, and then the motor/cup and pump must be aligned and fixed on a board so that the cup surrounds the magnetic unit in the pump. This task may be performed by someone with basic mechanical workshop competence. An example of the motor/pump combo is shown in Fig. 6b where Micropump GJ-N25 and motor M586TE are assembled together.

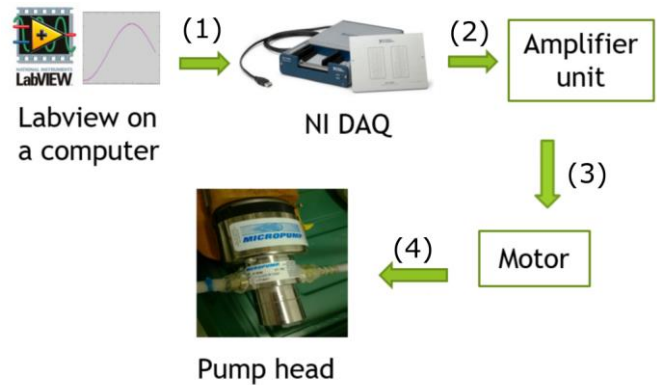


Fig. 7. The diagram for assembling a pump system

Normally the motor is controlled by voltage signals to allow changing the pump's output flow rate, which consequently can generate various pulsatile flow waveforms within the phantom. Variations of the control signals can be achieved from a computer where software such as LabVIEW is installed to design and output different waveforms of control signal (illustrated in Fig. 7). A data acquisition (DAQ) device which normally can output/receive control signal from/to computer via USB or PCI interface is required for communication between the computer and the pump system. The NI DAQ USB-6341 was used for the phantoms in this study. The level of the control signal for driving the motor is determined by the motor's power at working condition. A large pump with a high power requirement may not be able to be driven by signals directly from the output of the computer. In this case, a motor drive amplifier unit is needed. For example, the motor M586TE working at about 60W requires an amplifier unit before it can be driven by the output signal from a computer. A smaller pump, such as a GA-v21 Micropump, can be driven by control signals directly from the computer and in this case, the amplifier unit in Fig. 7 can be removed. The two pump systems can be chosen in different applications according to the required flow rate. The components of these two pump system are listed in Table VI. The signal flow at different stages in Fig. 7 are explained as below:

- 1) The control waveform was generated in LABVIEW with numerical values.
- 2) The numerical waveform was converted into a voltage signal waveform within -10 –10 V.
- 3) A power amplifier was needed to lift the level of voltage signal if the pump motor needed to work with a high power; this amplifier may not be required for a lower power pump motor.
- 4) The rotating motor drove the pump to generate pulsatile or constant flow in the phantom.

The capability to generate reverse flow is also an issue to be considered when choosing a pump system as some arterial flow waveforms include periods of reverse flow. This requires a pump capable of changing its working direction when the control signal changes its polarity. It is noted that some pumps are not designed to generate reverse flow.

TABLE VI.
PUMP SYSTEMS FOR FLOW PHANTOM

Equipment	Manufacturer	Details
GJ-N25 Micropump	Micropump, Vancouver, WA, USA	Flow rate from 158 ml/min to 6.8 L/min
GJ-N25 pump system	M586TE motor	Motor for driving the pump GJ-N25. 60 W Servo Motor, 36 V dc, 20Ncm, 3200 rpm.
	Power amplifier 4020-LS	Output control signal (-40 – 40V) for motor M586TE
GA-V21 pump system	GA-V21, Micropump	Flow rate from 8.5 ml/min to 506 ml/min
	Eagle Drive, Micropump	Motor for driving the pump GA-V21
NI DAQ USB-6341	National Instruments, Austin, USA	Control signal (-10V-10V) from the computer

V. BLOOD MIMICKING FLUID

BMF is needed both in the wall-less phantom and in the PVA-c vessel flow phantom, responsible for reflecting

ultrasonic sound waves for estimating flow velocity in a same way as in arteries. Physical properties such as volume concentration of acoustic backscatterer, scatterer size, fluid density, compressibility between the scatterer and the surrounding fluid, viscosity and acoustic properties have to be considered in the blood mimicking fluid (BMF) [8]. A recipe proposed by our group, using 5- μ m-diameter nylon particles (similar size to red cell in real blood fluid), was widely used in the literature and also quoted in IEC 61685[10]. Although this BMF is a Newtonian fluid, it has very close properties to human blood, with density of $1037 \pm 2 \text{ kg.m}^{-3}$, viscosity of $4.1 \pm 0.1 \text{ mPa.s}$, sound speed of 1548 m.s^{-1} and attenuation coefficient of $0.05 \pm 0.01 \text{ dB cm}^{-1} \text{ MHz}^{-1}$ over 3-10 MHz. More details about its properties can be found in the original paper [12].

Detailed procedure for making the BMF is described as follows and the equipment used is listed in Table VII and the used equipment is listed in Table VIII.

- 1) Measure out the DI water to a clean plastic beaker with the required amount. The beaker and measuring containers are washed clean with plenty of DI water. Wipe them with wipers. REMARKS: the volume of the plastic beaker must be over 2-fold of the required blood mimic. Failure to do this may cause the blood mimic to overflow while stirring.
- 2) Place a magnetic stirrer bar into the plastic beaker. Make sure to use a plastic beaker with a diameter about 5 - 7 cm bigger than the length of stirrer bar. REMARKS: If the diameter of beaker is too large, it could cause incomplete mixing during stirring.

TABLE VII.
RECIPE FOR MAKING BMF

Component	Manufacturer	Weight (%)
DI water	---	83.86
Glycerol (99%)	Sigma-Aldrich Ltd, Dorset, UK	10.06
5 μ m orgasol particles (2001 UD Nat 2)	Arkema Inc, 900 First Avenue, King of Prussia, PA 19406, USA	1.82
Dextran	Sigma-Aldrich Ltd, Dorset, UK	3.36
Synperonic N	Only available outside Europe market	0.9
Benzalkonium chloride (BC) 50% solution	Thermo Fisher Scientific, Geel, Belgium	1-2 drops

- 3) Weigh orgasol powder, dextran and glycerol in turn and tip them into the beaker. Try to decant all the required powder into the beaker.
- 4) Measure synperonic N, and also tip this into the plastic beaker. At this stage, the orgasol will not be wetted and

will sit on the top of the water. REMARKS: Do not try to stir the compounds in the beaker with any other tools to avoid losing powder.

- 5) Reseal chemical containers within the fume hood. Wipe down any contaminants using wet wiper and dispose of wipers appropriately. REMARKS: The particles used here are sufficiently small that they will stay in the human body for life if inhaled. Always remember to handle it carefully in the fume hood and wipe down any spilled powders with wet wipers.
- 6) If the BMF is needed for long term, 1-2 drops of BC 50% solution can be added to the BMF at this point.
- 7) Place the plastic beaker onto a magnetic stirrer plate. Set the speed of about 7 rotations per second. Leave for about 3 h for the powders to become wetted and in suspension in the liquid. Switch off fume hood when finished.
- 8) Filter the BMF with the 38 μm sieve. The sieve may become clogged and will need to be cleaned out from time to time with a small brush. REMARKS: Wipe down any leakages during the filtering.
- 9) Degas the BMF with the vacuum pump for about 2 – 3 h. REMARKS: For the first few minutes the air bubbles may threaten to overflow the beaker. If this happens release the vacuum slowly using the vent valve and turn it back after the bubbles settle down.
- 10) After the BFM has been degassed it is ready to use. The BMF may be stored for about 2 months and up to 6 months if antifungal agent was used. The BMF must be stored and sealed in a container when not in use, otherwise the water will evaporate and percentages of components will not be correct. Degassing has to be done again before usage of the BMF next time.

TABLE VIII
EQUIPMENT FOR PREPARING BMF

Equipment	Manufacturer/Distributor details
Fume hood	
Magnetic stirrer	SB302, Bibby Scientific Ltd (Stuart), Staffordshire, UK
Vacuum pump	KNF Neuberger D-79112, Vacuum Diaphragm Pump, Breisgau, Germany
38 μm sieve	59980-24, Cole-Parmer Testing Sieve, London, UK
Scale	PCB 2500-2, Kern & Sohn GmbH, Balingen, Germany
Others	Plastic beaker, disposable gloves, wet wipers

VI. RESULTS

A. Flow phantom examples

An example of a complete PVA-c vessel phantom system is shown in Fig. 8, where the phantom is being scanned by a clinical ultrasound scanner (HDI5000, Philips Medical Systems, Bothell, WA, USA). Figure 9 shows a KC-based wall-less flow phantom being scanned using a preclinical scanner (Vevo 770, FUJIFILM VisualSonics, Toronto, ON, Canada).

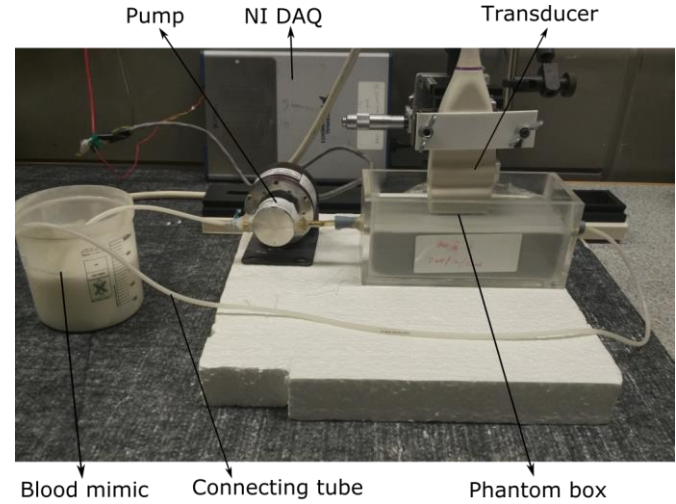


Fig. 8. An example of a complete flow phantom system used with a Philips HDI 5000 clinical scanner



Fig. 9. KC-TMM flow phantom used with the Visualsonics Vevo 770 preclinical scanner

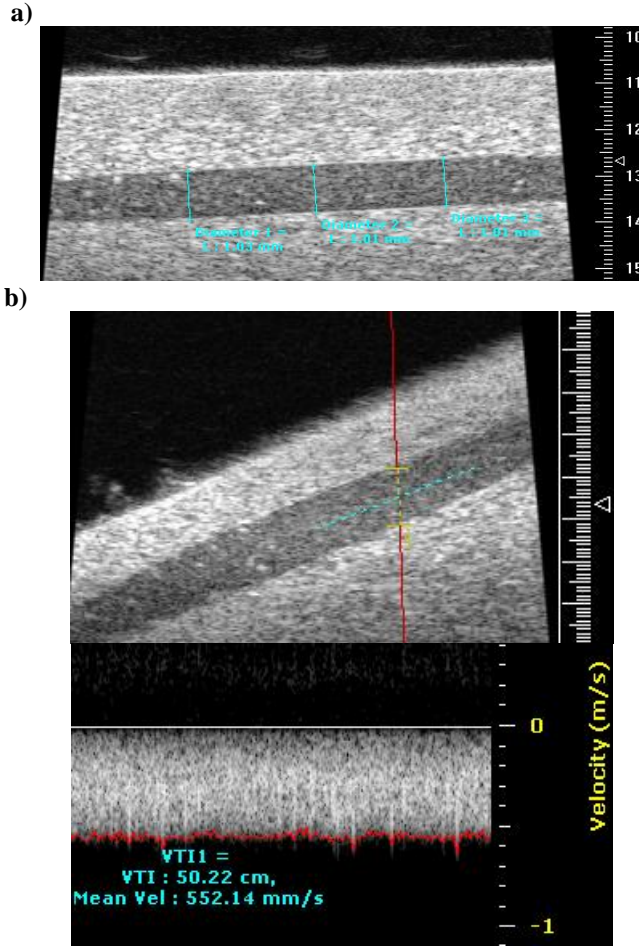


Fig. 10. Images from the KC-based wall-less phantom with steady flow made using a preclinical scanner. (a) Measurement of diameter from the B-mode image (transmit frequency of 23 MHz). Scale is mm. **Diameters are marked in blue (1.03mm, 1.01mm, 1.01mm)**; (b) Measurement of maximum velocity from spectral Doppler (prf of 20 kHz). B-mode image scale is mm.

B. Images from KC-based vessel-less flow phantom

A Vevo 770 ultrasound scanner with a 710B transducer (bandwidth 15 – 25MHz) (FUJIFILM VisualSonics, Toronto, ON, Canada) was used to scan a well-fabricated wall-less flow phantom. The diameter of the lumen is about 1 mm for this flow phantom. B-mode image and images from pulsed-waved spectral Doppler mode are presented in Fig. 10.

C. Images from PVA-c vessel flow phantom

An HDI 5000 clinical ultrasound scanner (Philips Medical Systems, Bothell, WA, USA) with a L12-5 transducer was used to scan the PVA-c vessel flow phantom, fabricated according to the methods presented herein, where the vessel diameter is 2.6 mm. The images acquired from this scanner are shown in Fig. 11, including the B-mode images, Colour Doppler images and the spectral Doppler images.

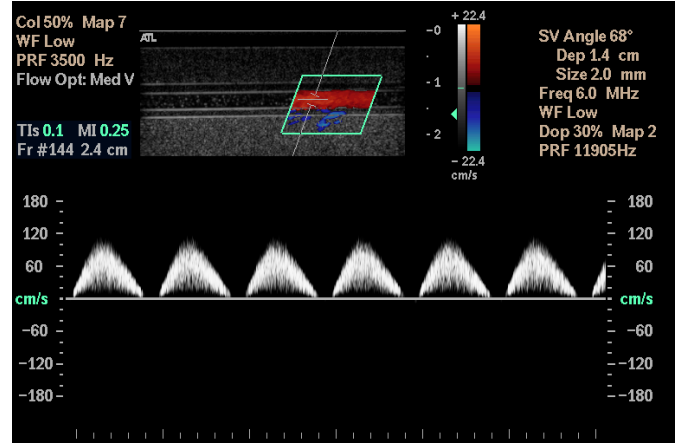


Fig. 11. Images from the PVA-c vessel flow phantom with pulsatile flow at 1 Hz using a clinical scanner. Transmit frequency is 6 MHz and prf is 11905 Hz.

VII. DISCUSSION

A. Pump system

The flow phantoms described in this paper are based on use of gear-pumps. The original gear-pump flow phantoms were developed in Edinburgh; the initial system designed mainly for steady flow use [23], and the second system [24] with computer control of pump speed allowing production of pulsatile flow. Over the subsequent 27 years gear-pump based flow phantoms have been used in over 20 publications from Edinburgh; eg. [13, 15, 17, 22] and adopted by other groups. The gear pump has a number of features which make it ideal for the generation of pulsatile flow including rapid temporal response, lack of backflow and ability to control pump speed by an external electronic signal. Gear pumps are available which provide a range of maximum flow output; enabling manufacture of flow phantoms suiting a wide range of flow rates from a few ml per minute (preclinical) to hundreds of ml per minute (clinical).

As noted above control of pump speed allows the production of time-varying or pulsatile flow. However the flow pulse is damped between the pump and the phantom due to the elastic nature of the connecting tubing, and there are reflected waves from junctions in the connecting tubing, especially at the end of the tubing. Measures to reduce reflected waves include the use of high resistance (low diameter) distal vessels, however these also increase fluid pressure which may exacerbate rupture. In practice the control waveform is adjusted until a desired Doppler waveform is obtained. These issues are further discussed in [8]. The use of a flowmeter (eg. Transonics Inc., NY, USA), immediately distal from or proximal to the phantom can be used to gain knowledge of the flow waveform.

B. KC-based wall-less flow phantom

As noted in section I the KC-TMM wall-less phantom was originally developed to overcome problems of rupture which occurred when the IEC compliant agar-based TMM was used [14]. In more recent preclinical applications [15] the KC-TMM was found to be an ideal candidate for a wall-less phantom,

tested for depths as low as 1 mm between the surface of the KC-TMM and the vessel cavity.

The disadvantage of the KC-TMM over the agar-based TMM is that it is not suitable for long term use or storage. Use of a preservative such as benzalkonium chloride caused major alterations to the acoustic properties of the KC-TMM. Whereas the agar-based TMM does contain benzalkonium chloride, the KC-TMM does not and is susceptible to mold growth. This could not only affect the acoustic properties, but also affect the free passage of the BMF through the vessel. Therefore it is recommended that on the appearance of mold on the surface of the KC-TMM, the KC-TMM should be discarded.

The B-mode appearance of the KC-TMM was similar to that from the agar based TMM in that there was a speckle pattern which was very similar to that seen in the B-mode images of tissues.

C. PVA-c vessel flow phantom

The distinct feature of the PVA-c vessel phantom described here is that it creates the physiologically equivalent environment for Doppler ultrasound imaging in the arterial applications. This well-fabricated phantom can be used both for mimicking the flow within the vessel and also for mimicking the motion characteristics of the vessel wall [17]. Recipes for these mimicking materials were carefully chosen and their physical properties have been proved by previously published studies. As shown in Fig. 11, images from the phantom show clear visualisation of vessel wall boundaries allowing studies on wall motion, and both colour flow and spectral Doppler allowing study of blood velocity measurements.

The TMM surrounding the vessel could give rise to vessel non-circularity due to the effect of the surrounding TMM. Measures taken to minimize non-circularity included burying the vessel at only a shallow depth within TMM, making the PVA vessel wall thicker and filling the vessel with water while pouring the TMM. However in practice there remained a small difference between the horizontal and vertical vessel diameters.

Use of the KC-TMM with a PVA-c vessel was not possible as the high temperature (80 °C) at which the liquid TMM is poured would destroy the PVA-c vessel.

A pure PVA-c vessel does not have scatterers and does not provide acoustic scatter, hence appears dark on the B-mode image. In this study, 0.75% Silicon carbide was added to generate backscatter from the PVA-c vessel mimic.

D. Overall

Flow phantoms are designed to match key features of the vessels of interest. These features include diameter, depth, flow rate, blood velocity, wall motion and flow waveform shape. More complex features include vessel geometry (e.g. curvature, bifurcation) and disease (e.g. inclusion of a stenosis). As noted above a key feature is acoustic matching of phantom properties to the acoustic properties of tissues. Rat and mouse arteries are typically 5 and 10 times less than those of human arteries (e.g. typical common carotid artery diameter; human 5 mm, rat 1 mm, mouse 0.5 mm), and are also located closer to the skin surface than in the human. For this reason flow phantoms

designed for clinical (human) use are unsuitable for preclinical use.

The two flow phantoms provide very close acoustic matching to soft tissue, arterial wall and blood, which is essential in studies on validation of key measured quantities such as diameter, maximum velocity, mean velocity, wall shear rate and volumetric flow [15, 25]. The sizes of the vessel, vessel depth in the TMM, flow rate and the flow waveform can all be adapted to suit different applications. For clinical use typical vessel diameters (mimicking human vessels) are 3-10mm. For preclinical applications vessel diameters are typically 0.5-2mm. The dimensions of the corresponding phantom are determined by a combination of inlet-length requirements (to produce fully-developed flow [8]) and by practical issues concerned with allowing sufficient access for the transducer to make contact with the TMM. For clinical use the maximum phantom dimension is typically 15-30cm, for preclinical it is 5-10cm. In general preclinical flow-phantom dimensions are less than clinical flow-phantom dimensions due to the lower inlet length required to produce stable flow (inlet length is proportional to vessel diameter squared) and due to the smaller footprint of preclinical transducers compared to that of clinical transducers.

The main future issues to address are concerned with production of anatomical models, simulation of arterial disease, refinement of blood mimics to account for non-Newtonian behavior and validation of velocity measurements against an independent technique such as particle image velocimetry [7].

Undoubtedly a key technology for future work on phantom development in general and for flow phantoms in particular is 3D printing. There have been a tiny number of papers describing manufacture of flow phantoms using 3D printing [e.g. 14, 26-28]. These studies involve a number of steps; 3D printing is just one step in a series of steps towards the final phantom. For example in [14] and [26] the steps are: production of a solid core in the shape of an artery using 3D printing; production of a mould from the solid core; production of a low melting point alloy core from the mould; incorporation of the lost core into a box; pouring and setting of the TMM; melting of the alloy to produce the final phantom. The goal in this area would be the generation of the complete phantom using 3D printing. There remains significant work to do in this area, mainly around the formulation of a material which is both 3D-printable and acoustically equivalent.

VIII. CONCLUSION

The comprehensively detailed procedures for preparation of phantom materials and fabrication of the KC-based wall-less flow phantom and the PVA-c vessel flow phantom in this paper can be helpful to researchers who require the knowledge to fabricate flow phantoms optimally designed for their particular Doppler ultrasound measurements.

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